



Heterocyclic Amine Content of Pork Products Cooked by Different Methods and to Varying Degrees of Doneness

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Abstract—Heterocyclic amines (HCAs) are known mutagens and animal carcinogens produced in meats cooked at high temperature. As pork is the second most frequently consumed meat in the United States, five predominant HCAs [2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)] were measured in various pork products, cooked by different techniques and to varying doneness levels. Pork chops and ham slices were pan-fried and oven-broiled; bacon was pan-fried, oven-broiled or microwaved; hot dogs were pan-fried, oven-broiled, grilled/barbecued or boiled; sausage links and patties were pan-fried. All the products were cooked to three levels of doneness: just until done, well done or very well done. HCA type and level varied substantially by pork product, cooking method and doneness level. The highest PhIP levels were found in well done and very well done oven-broiled bacon; for very well done 30.3 and 4.0 ng per gram of meat of PhIP and MeIQx, respectively. Pan-fried very well done sausage patties contained 5.4 ng of MeIQx per gram of meat, while sausage links contained 1.3 ng per gram of meat. MeIQx was formed in well done and very well done pan-fried but not broiled pork chops. Hot dogs or ham slices had low or undetectable levels of HCAs. These results demonstrate that epidemiological studies investigating the relationship between HCA intake and cancer risk need to incorporate type of meat, cooking method and degree of doneness/surface browning into questions to assess adequately an individual's HCA exposure. © 1998 Elsevier Science Ltd. All rights reserved

Abbreviations: HCAs = heterocyclic amines; MeIQx = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; DiMeIQx = 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline.

Keywords: heterocyclic amines; pork; pan-fry; oven-broil; grill; barbecue; microwave; MeIQx; DiMeIQx; PhIP; IQ; MeIQ; meat; hot dog; sausage; pork chop; ham steak.

INTRODUCTION

Heterocyclic amines (HCAs) are formed in meats cooked at high temperatures. HCAs are potent mutagens and animal carcinogens (Adamson, 1990; Felton and Knize, 1990; Ghoshal *et al.*, 1994; Ito *et al.*, 1991; Ohgaki *et al.*, 1986; Skog, 1993;

Weisburger *et al.*, 1994). However, the carcinogenic potential in humans is yet to be established (Steineck *et al.*, 1993). Epidemiological studies of colon and breast cancer using crude surrogates for HCA exposure (e.g. doneness, surface browning, frying, intake of gravy) have produced suggestive but somewhat inconsistent results (Gerhardsson De Verdier *et al.*, 1991; Knekt *et al.*, 1994; Muscat and Wynder, 1994; Ronco *et al.*, 1996; Schiffman *et al.*, 1990; Steineck *et al.*, 1993). Currently used

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surrogates such as doneness of 'red meat' may be inadequate to assess an individual's exposure to HCAs since substantial heterogeneity of HCA levels exists in a variety of meats all considered as 'well done' (Sinha and Rothman, 1996). The misclassification may result in decreased ability to observe a true association of HCA and cancer risk. Thus, to decrease misclassification and better assess the contribution of HCAs in cancer aetiology there is a need to improve exposure assessment of HCAs. To this end, we are developing a database of HCA concentrations in commonly consumed meat items cooked by various techniques to various degrees of doneness (Knize *et al.*, 1995 and 1996; Sinha *et al.*, 1995 and 1998).

Pork is the second most frequently consumed meat in the US (National Live Stock and Meat Board, 1994), and could contribute to HCA exposure in the general population. Cooking techniques and the degree of doneness (Berg *et al.*, 1990; Gross *et al.*, 1993; Johansson and Jagerstad, 1994) influences HCA content in pork products. Here we report the HCA content of some of the most commonly consumed pork products: bacon, pork chops, ham slices, sausage links and patties, hot dogs and bacon drippings cooked by different methods to varying degrees of doneness. These measurements will provide data to accurately assess HCA exposure in epidemiological studies.

MATERIALS AND METHODS

Five pork products were purchased from a local supermarket: bacon (regular sliced); sausages (patties, regular links and fully cooked and pre-browned links or 'brown-n-serve'); hot dogs; pork chops; and ham slices. The pork products were cooked by commonly used techniques for that particular meat. Bacon was pan-fried, oven-broiled or microwaved; hot dogs were pan-fried, oven-broiled, grilled/barbecued or boiled; sausage was pan-fried; pork chops and ham slices were pan-fried or oven-broiled.

Nutritionists at the Human Nutrition Research Center, United States Department of Agriculture, Beltsville, MD cooked the pork products. Each cooking method and doneness level for a particular meat item was repeated in multiple sessions. Details on the number of samples cooked in each session and information on cooking conditions are provided in Appendices 1–5. The cooked meat was finely ground in a Robot Coupe mixer (Jackson, MS, USA) to form a composite sample for the specific meat type, method of cooking and degree of doneness.

The bacon, sausage, hot dogs, pork chops and ham slices were cooked to three degrees of doneness: just until done, well-done and very well done/crisp. The degree of doneness for sausage, hot dog, pork chops and ham slices was defined primarily by

internal temperature which was taken using a tissue implantable thermocouple microprobe (type 1T-18, Physitemp Instruments, Inc., Clifton, NJ, USA) connected to a base thermocouple digital thermometer (Model no. 08500-40, Cole-Parmer, Chicago, IL, USA). We defined pork cooked to an internal temperature of 70°C as just until done, 80°C as well done, and 90°C as very well done. The interior colour was noted by visual inspection of each sample. The level of surface browning was judged to be one of the following categories: not browned; well browned; and very well browned/charred. For bacon, the degree of doneness was based primarily on visual inspection. Photographs of each doneness category were taken to show the external colour (Plates 1–4).

We used the cooking methods most commonly used in the US: pan-fried, oven-broiled and microwaved. Bacon samples were pan-fried in a teflon-coated frying pan without added oil. The temperature on the pan surface was monitored with a surface thermometer (PTC, Pacific Transducer Corp., Los Angeles, CA, USA). The bacon was turned as often as necessary after 2 min of cooking. For well done and very well done bacon the fat released during cooking was removed at approximately 4 min of cooking. Bacon slices were oven-broiled 12 cm away from the heat source in a commercial gas range and turned every 2 min until done. The surface temperature was monitored with a thermocouple probe near the surface of the broiling pan. Bacon slices were microwaved on absorbant paper in an institutional grade microwave (Litton MenuMaster, System 80142) until the level of brownness was comparable to the pan-fried bacon. Sausage links and patties were pan-fried as described earlier. 'Brown-n-serve' sausages were cooked in a small amount of water in a frying pan. Pork chops and ham slices were pan-fried or oven-broiled. Hot dogs were pan-fried, oven-broiled, boiled or grilled. Grilled hot dogs were prepared on a gas barbecue unit with ceramic briquettes (Sunbeam model 44M39 27.5 × 15 in., 44,000 BTU) sold for home use. The surface temperature was monitored with thermometers on the grill surface.

Detailed information on criteria used to classify doneness, such as internal temperature and surface browning, is shown in Appendices 1–5. Other information gathered to further define the cooking methods included: weight of meat before and after cooking to calculate the percent loss of weight with cooking; and the total cooking time.

The levels of 2-amino-3-methylimidazo[4,5-*f*]-quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]-quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) were measured in duplicate samples by solid-phase extraction and analysed by reverse

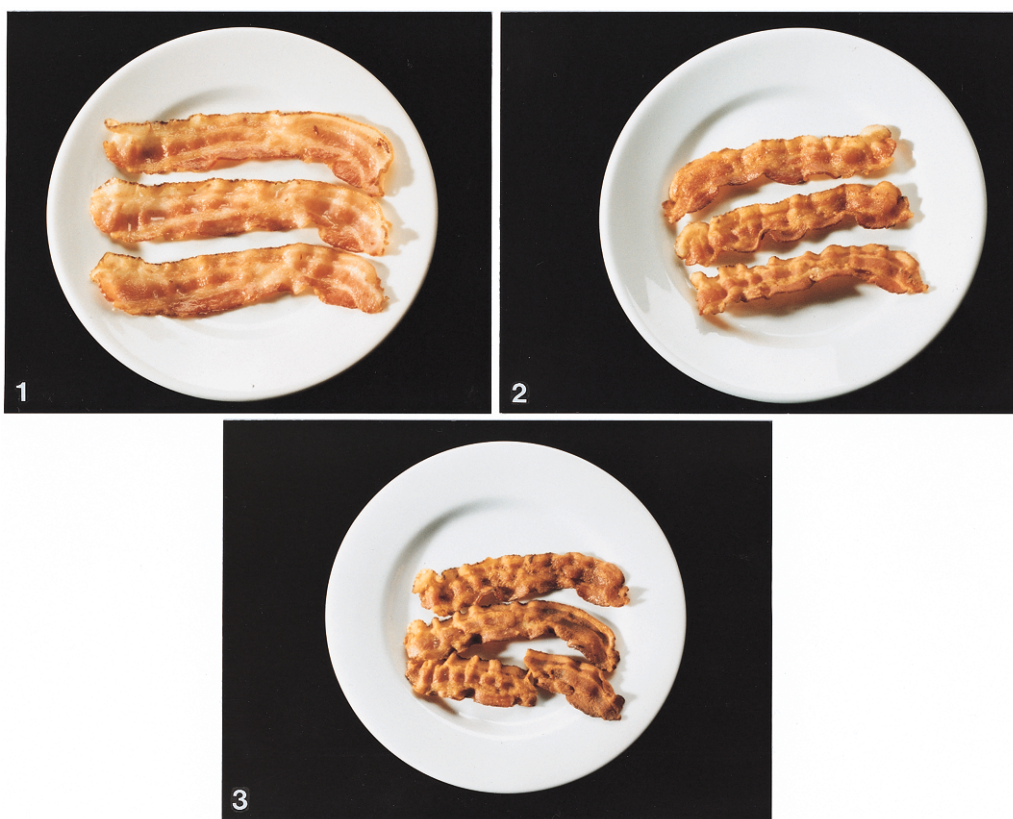


Plate 1. Appearance of bacon pan-fried to three levels of doneness.

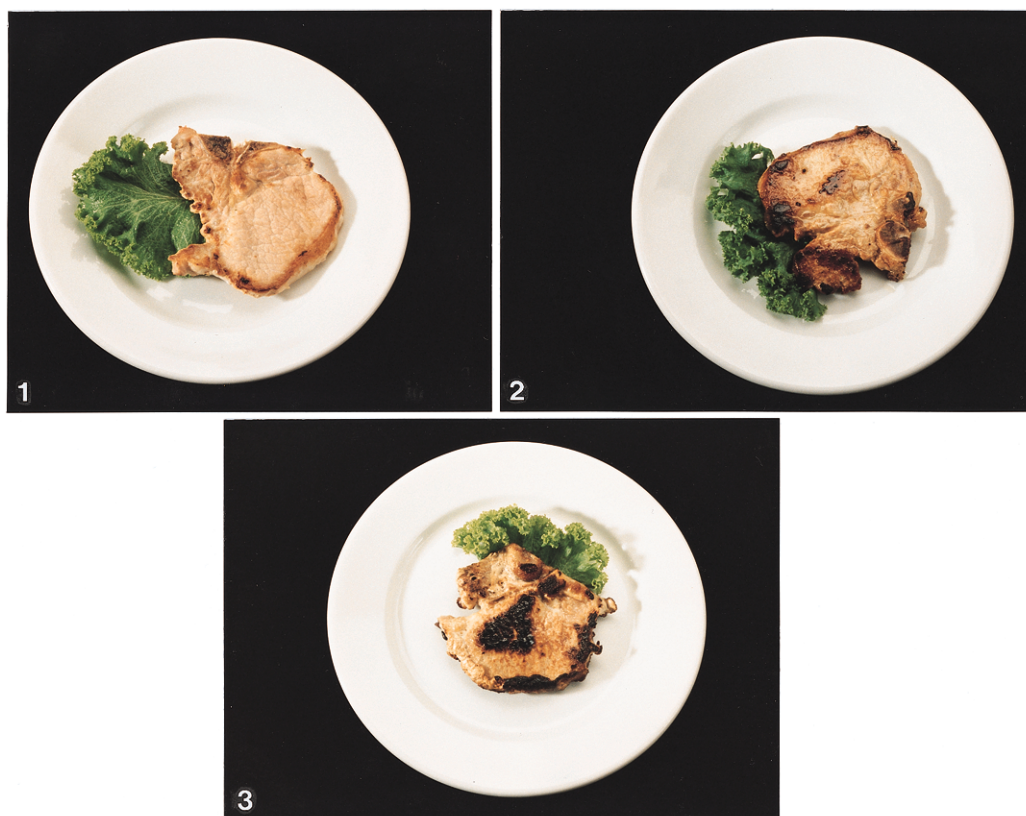


Plate 2. External appearance of pork chops pan-fried to three levels of doneness: 1—just until done; 2—well done; 3—very well done.



Plate 3. External appearance of ham slices pan-fried to three levels of doneness: 1—just until done; 2—well done; 3—very well done.

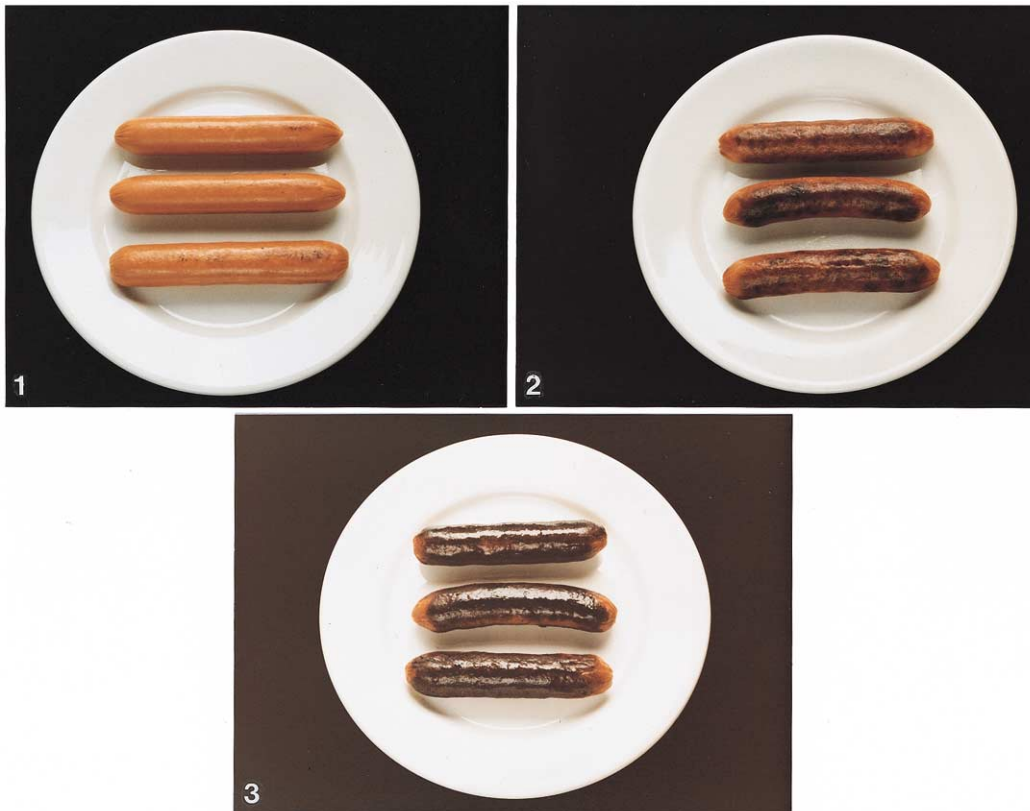


Plate 4. External appearance of hot dogs pan-fried to three levels of doneness: 1—just until done; 2—well done; 3—very well done.

phase HPLC (Gross and Gruter, 1992; Knize *et al.*, 1995). HCA recoveries for each sample were determined from the average of duplicate samples spiked with all five compounds. The identities of peaks at the retention time of known HCAs were confirmed by photodiode-array UV spectra in all cases (Knize *et al.*, 1995). The investigators measuring HCA content were blinded to the type of meat, cooking method and degree of doneness. Quality control samples with relatively low and high content of HCAs were interspersed throughout the analysis to check on measurement reproducibility. These samples were made from hamburger cooked at low temperature (containing low levels of HCAs) or hamburger patties cooked at high temperature (containing high levels of HCAs). The average concentrations of MeIQx, PhIP, and DiMeIQx found in the high-temperature samples ($n = 13$) were: 7.2 (coefficient of variation: 0.36), 10.9 (0.24) and 1.7 (0.40) ng/g, respectively (Knize *et al.*, 1995). IQ and MeIQ were not present in the high temperature quality control samples and none of the five HCAs were found in the low temperature quality control samples. The limit of detection was approximately 0.2 ng/gram of cooked meat for all five compounds.

RESULTS

Plates 1–4 show pork products cooked to three levels of doneness. Figure 1 presents the concentrations of MeIQx and PhIP in bacon samples cooked by different methods to varying degrees of doneness. In general, both MeIQx and PhIP levels were higher in very well done and well done bacon than in samples cooked just until done. Oven-broiled bacon contained high levels of PhIP compared with bacon cooked by other methods. Even bacon cooked to a very well done level in the microwave oven contained both MeIQx and PhIP, while IQ, MeIQ and DiMeIQx were not detectable in any of the bacon samples. Bacon fat from pan-fried very well done bacon samples (Table 1) had minimal levels of MeIQx but did contain some PhIP (2.3 ng/g fat).

HCA content of all other types of pork products are presented in Table 1. Very well done sausage links contained 1.3 ng of MeIQx per gram of meat, while similarly cooked patties contained 5.4 ng of MeIQx per gram of meat. Pan-fried ham slices and pork chops contained some MeIQx, while oven-broiled products had non-detectable levels of all five HCAs. None of the hot dogs samples had detectable levels of HCAs.

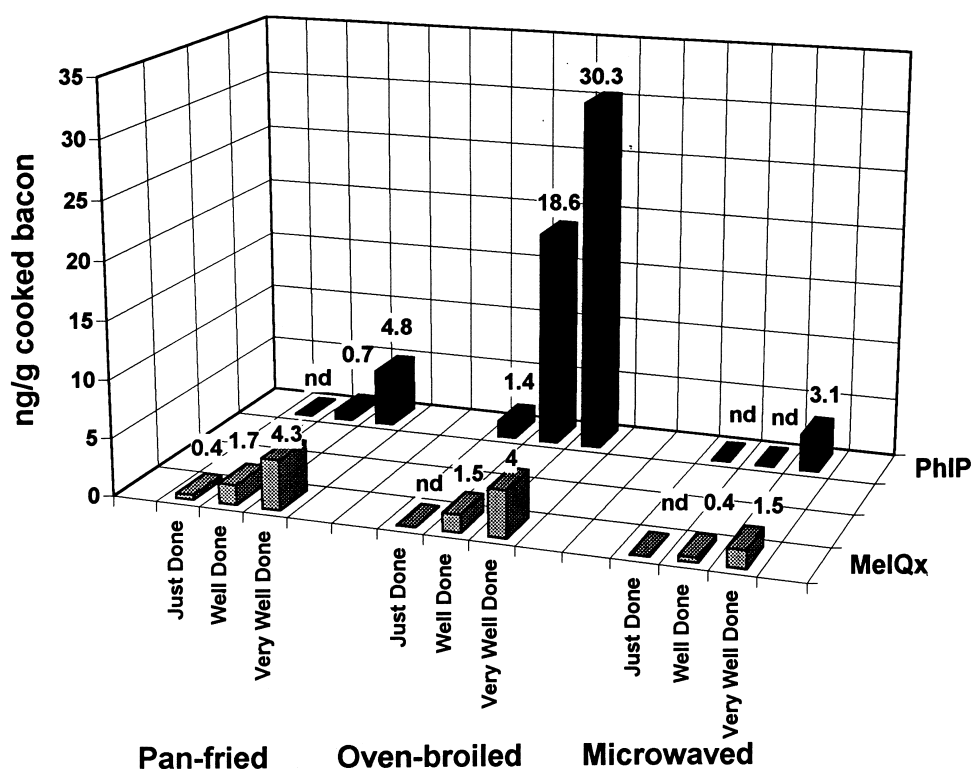


Fig. 1. Mean MeIQx and PhIP content of bacon cooked by different methods to varying degrees of doneness.

Table 1. MeIQx and PhIP content of cooked pork products

Type of meat and cooking method and degree of doneness	MeIQx (ng/g meat)	PhIP (ng/g meat)
<i>Sausage links—pan-fried</i>		
Just until done	nd	nd
Well done	0.4	nd
Very well done	1.3	0.1
<i>Sausage patties—pan-fried</i>		
Just until done	nd	nd
Well done	1.6	nd
Very well done	5.4	nd
<i>Ham slice—pan-fried</i>		
Just until done	nd	nd
Well done	0.6	0.3
Very well done	1.8	nd
<i>Pork chop—pan-fried</i>		
Just until done	nd	nd
Well done	1.3	nd
Very well done	3.8	nd
<i>Bacon fat—pan-fried</i>		
Just until done	nd	nd
Well done	nd	nd
Very well done	0.6	2.3
<i>Hot dog—pan-fried</i>		
Just until done	nd	nd
Well done	nd	nd
Very well done	nd	nd
<i>Hot dog—oven-broiled</i>		
Just until done	nd	nd
Well done	nd	nd
Very well done	nd	nd
<i>Hot dog—grill/barbecued</i>		
Just until done	nd	nd
Well done	nd	nd
Very well done	nd	nd
<i>Hot dog—boiled</i>		
Well done	nd	nd

nd = not detectable

DISCUSSION

HCA values for various pork products cooked by different methods and to different level of doneness are presented so that they can be used in calculating exposure to these compounds in epidemiological studies. Bacon contained the highest levels of PhIP while the other pork products had undetectable or low levels of HCAs. Bacon is different from other meats in that oven broiling produced relatively high levels of PhIP compared with that produced by other cooking methods. In contrast, oven-broiled beef or chicken had lower levels of PhIP and MeIQx than pan-fried or grilled/barbecued samples (Sinha *et al.*, 1995 and 1998).

Our results agree with several published studies for bacon. Gross *et al.* (1993) reported detectable levels of MeIQx in all cooked bacon samples tested, up to 18 ng/g, while PhIP was detected only in some samples but was as high as 53 ng/g. Skog *et al.* (1995) found 0.3–4.5 ng PhIP/g, and MeIQx in some bacon samples, up to 23.7 ng/g. Johansson and Jagerstad (1994) detected MeIQx and DiMeIQx in all bacon samples; with low levels of PhIP in two of four samples, however, in contrast to our study, IQ and MeIQ were also detected.

The high levels of PhIP in bacon raises several interesting issues in relation to HCA intake and cancer aetiology. Data from the Continuing Survey

of Foods II (US Department of Agriculture, 1986) indicate that bacon was the most frequently consumed meat item in the US. Further, there are two reports that indicate that HCAs in bacon are bio-available and bacon consumption is positively associated with urinary excretion of MeIQx, especially among African Americans (Ji *et al.*, 1994; Stillwell *et al.*, 1994). Thus, the high levels of PhIP in oven-broiled bacon indicate that people consuming bacon frequently, especially if it is cooked very well done, may have a high exposure to HCAs. Moreover, using bacon fat in cooking would further add to the amount of HCAs in the diet.

Even though pork is the second highest meat consumed in the US (American Meat Science Association, 1978), in absolute terms most of the pork products did not contain very large amounts of HCAs, especially when compared with beef and chicken. In a previously reported study, very well done pan-fried hamburgers cooked to maximize the production of these compounds contained 32.8 ng/g PhIP (Sinha *et al.*, 1994). The comparable preparation of pan-fried, very well done chicken breast contained more than twice the amount of PhIP at 70 ng/g meat (Sinha *et al.*, 1995). Compared with pan-fried hamburger patties and chicken, PhIP levels for pork chops, ham slices, sausage (link or patty) or hot dogs were non-detectable. Bacon did contain higher levels of PhIP but only in the oven-broiled samples. MeIQx levels in the various pork products were in a similar range to those measured in the beef and chicken samples.

The production of HCAs may depend on the shape of the meat product. For example, only 1.3 ng MeIQx per gram of meat is formed in sausage links compared with 5.4 ng/g of meat in sausage patties that were pan-fried. The difference in HCA production may be due to various reasons. Patties are flat with a larger surface area in contact with the hot surface. Moreover, in sausage links, meat juices containing HCA precursors remain inside the casing and do not come in contact with the hot surface except when the casing is broken.

Interestingly, hot dogs had non-detectable levels of HCAs even when cooked to very well done, and cooking method did not influence the production of these compounds. This could again be due to the shape, similar to sausage links, and the containment of the meat juices. It may also be that the meat in hot dogs is processed and contains very low levels of HCA precursors normally present in fresh meat. Although we failed to find HCAs in pork hot dogs, high levels of mutagenic activity, presumed to be from HCAs, were reported in some grilled hot dogs (Stavric *et al.*, 1995).

The data provided here will help in creating an exposure index for HCAs, which can be used to categorize subjects in epidemiological studies,

especially using the photographs (Plates 1–4). To date, only surrogates of HCAs exposure (doneness or brownness of red meat) have been used in epidemiological studies. All types of meat, that is, beef, pork, chicken and fish, need to be incorporated in a matrix with cooking method and level of doneness in order to comprehensively assess total HCA exposure. The pork data are part of a larger database for meats cooked by different methods to varying levels of doneness which can be linked with a meat cooking module within a food frequency questionnaire (Sinha and Rothman, 1996).

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Appendix 1. Bacon cooked by three different methods to varying degrees of doneness: mean percent weight loss, cooking surface temperature, total cooking time and appearance

Cooking method and degree of doneness	Total slices (replicates × samples)	Weight loss during cooking (%)	Cooking surface temp. (°C)	Total cooking time (min)	Appearance
<i>Pan-fried</i>					
Just until done	80 (8 × 10)	69	176	4.0	pink
Well done	80 (8 × 10)	79	177	8.8	slightly brown
Very well done	80 (8 × 10)	79	176	16.1	brown and crisp
<i>Oven-broiled</i>					
Just until done	80 (8 × 10)	71	185	4.7	pink
Well done	80 (8 × 10)	76	175	5.4	slightly brown
Very well done	80 (8 × 10)	77	175	7.2	brown and crisp
<i>Microwaved</i>					
Just until done	80 (5 × 16)	63	not determined	1.8	pink
Well done	80 (5 × 16)	76	not determined	2.5	slightly brown
Very well done	80 (5 × 16)	77	not determined	3.3	brown and crisp

Fresh regular sliced bacon purchased from a local supermarket.

Pan-fried: fried in a teflon pan without added oil. Turned at 2 min and more often for well and very well done. Fat removed at around 4 min for well done and very well done samples.

Oven-broiled: broiled 12 cm below the heated surface. Turned at approximately 3 and 5 min.

Microwaved: microwaved in a Litton MenuMaster system 80/42, 1050 W, 4000 V, 2450 MHz.

Appendix 2. Hot dogs cooked by four different methods to varying degrees of doneness: mean percent weight loss, cooking surface and internal temperature, total cooking time and surface appearance

Cooking method and degree of doneness	n (replicates × samples)	Weight loss during cooking (%)	Cooking surface temp. (°C)	Internal temp. (°C)	Cooking time (min)	Surface appearance
<i>Pan-fried</i>						
Just until done	20 (5 × 4)	2	175	54	4	pink
Well done	20 (5 × 4)	6	177	78	9	brown
Very well done	20 (5 × 4)	11	177	98	18	black
<i>Oven-broiled</i>						
Just until done	20 (5 × 4)	4	180	58	3	pink
Well done	20 (5 × 4)	12	182	81	6	brown
Very well done	20 (5 × 4)	20	185	100	10	black
<i>Grill/barbecued</i>						
Just until done	20 (5 × 4)	3	232	62	5	pink
Well done	20 (5 × 4)	7	260	82	8	brown
Very well done	20 (5 × 4)	19	252	100	15	black
<i>Boiled</i>						
Well done	20 (5 × 4)	2	100	72	5	pink

A national brand of hot dogs made with both pork and turkey was purchased from a local supermarket.

Pan-fried: fried in a teflon pan with 1 tablespoon vegetable oil. One quarter-turn every 30 sec until evenly browned.

Oven-broiled: broiled 12 cm below the heated surface. Turned at approximately 1.5 min for just until done, 3 min for well done and 5 min for very well done.

Grilled/barbecued: grilled on a home propane gas barbecue unit with ceramic briquettes (Sunbeam model 44M39 27.5 in. × 5 in., 44,000 BTU). One quarter-turn every 1 min until evenly browned.

Boiled: submerged in boiling water until internal temperature reached 68–79°C.

Appendix 3. Sausage pattie, regular links and brown-n-serve links pan-fried to varying degrees of doneness: mean percent weight loss, cooking surface and internal temperature, total cooking time and surface appearance

Cooking method and degree of doneness	n (replicates × samples)	Weight loss during cooking (%)	Cooking surface temp. (°C)	Internal temp. (°C)	Cooking time (min)	Surface appearance
<i>Regular links</i>						
Just until done	60 (5 × 12)	24	175	86	9	no brown
Well done	60 (5 × 12)	33	177	97	15	slightly brown
Very well done	60 (5 × 12)	41	177	100	21	well browned
<i>Pattie</i>						
Just until done	40 (5 × 8)	24	175	80	8	no brown
Well done	40 (5 × 8)	34	176	90	14	slightly brown
Very well done	40 (5 × 8)	45	179	99	21	well browned
<i>Brown-n-serve links</i>						
Well done	60 (5 × 12)	6	100	69	5	slightly browned

National brands of pork sausage links (2 oz per 24 links), pork sausage patties (12 oz per nine patties), brown-n-serve fully cooked, pre-browned pork links were purchased fresh from a local supermarket.

Links: fried in a teflon pan without added oil. Turned every 2 min until evenly browned. Fat removed after 10 min for well and very well done samples.

Patties: fried in a teflon pan without added oil. Turned at approximately 4 min for just until done, 7 min for well done and 10 min for very well done samples. Fat removed after 10–12 min for well and very well done samples.

Brown-n-serve: cooked per instructions on the package. Links placed in an unheated skillet with 2 tablespoons water and simmered for 5 min, then drained on paper towel.

Appendix 4. Pork chops cooked by two different methods to varying degrees of doneness: mean percent weight loss, cooking surface and internal temperature, total cooking time and surface appearance

Cooking method and degree of doneness	n (replicates × samples)	Weight loss during cooking (%)	Cooking surface temp. (°C)	Internal temp. (°C)	Cooking time (min)	Surface appearance
<i>Pan-fried</i>						
Just until done	20 (5 × 4)	17	175	75	5	no brown
Well done	20 (5 × 4)	27	176	82	9	some brown
Very well done	20 (5 × 4)	37	176	91	15	well browned
<i>Oven-broiled</i>						
Just until done	20 (5 × 4)	23	175	72	5	no brown
Well done	20 (5 × 4)	34	189	83	7	some brown
Very well done	20 (5 × 4)	45	179	98	11	well browned

Fresh 0.5 in. centre in loin pork chop with bone was purchased from a local supermarket.

Pan-fried: fried in a teflon pan without 1 tablespoon vegetable oil. Turned at approximately 3 min for just until done, 5 min for well done, and 10 min for very well done samples.

Oven-broiled: broiled 12 cm below the heated surface. Turned at approximately 2 min for just until done, 4 min for well done and 7 min for very well done samples.

Appendix 5. Ham slices cooked by two different methods to varying degrees of doneness: mean percent weight loss, cooking surface and internal temperature, total cooking time and surface appearance

Cooking method and degree of doneness	n (replicates × samples)	Weight loss during cooking (%)	Cooking surface temp. (°C)	Internal temp. (°C)	Cooking time (min)	Surface appearance
<i>Pan-fried</i>						
Just until done	5 (5 × 1)	18	175	73	5	pink
Well done	5 (5 × 1)	32	176	79	12	slightly brown
Very well done	5 (5 × 1)	37	176	86	19	charred
<i>Oven-broiled</i>						
Just until done	4 (4 × 1)	18	173	72	4	pink
Well done	4 (4 × 1)	38	189	86	9	slightly brown
Very well done	4 (4 × 1)	49	186	96	13	charred

Fresh, fully cooked, smoked ham slice (1–1.3 cm) with added water was purchased from a local supermarket.

Pan-fried: fried in a teflon pan without added oil. Turned at approximately 3 min for just until done, 7 min for well done, and 12 min for very well done samples.

Oven-broiled: broiled 12 cm below the heated surface. Turned at approximately 2 min for just until done, 5 min for well done and 8 min for very well done samples.